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# Development of a Novel Environmental Preference Test System for Laying Hens and Its Initial Application to Assess Hen Aversion to Atmospheric Ammonia

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## **Development of a Novel Environmental Preference Test System for Laying Hens and Its Initial Application to Assess Hen Aversion to Atmospheric Ammonia**

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**Abstract.** *An environmental preference test chamber (EPTC) was designed, constructed, and utilized in an initial test for response of laying hens to atmospheric ammonia. The EPTC featured four interconnected, individually ventilated clear acrylic compartments. Each compartment contained a wire-mesh cage that was divided into two sections, one section used for a test bird to navigate between the compartments and the other section used for three stimulus birds to reside in each compartment. The EPTC was designed to assess individual bird preference without isolation effects. The section dividers may be removed to assess group preference. An initial experiment was conducted with six test hens to assess bird aversion to atmospheric ammonia. Each hen was trained to navigate the inter-compartment door prior to the aversion test. Following one day of acclimation to the chamber, behavioral data of the hen were collected for 2 days at ambient conditions (baseline) and 3 days at ammonia level of 25 ppm vs. <10 ppm. Hen location (compartment) was documented and compared for baseline and treatment periods. All hens learned to navigate the chamber within 10 h; 4 of the 6 hens learned within 2 h. No preference for the level of ammoniated condition was observed with regard to occupancy of the corresponding compartment or number of entries into each environment. Further investigation is warranted to determine if this finding is a lack of aversion or other phenomenon. The EPTC will also enable future users to examine preference responses of hens to other environmental conditions, such as thermal comfort vs. air quality.*

**Keywords.** ventilation, air quality, aversion, behavior

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## Introduction

In recent years, the perceptions of laying hens regarding their housing environment have become an important factor for determining housing conditions and establishing husbandry guidelines, especially in the European Union (EU) (EFSA, 2005). Preference and motivation testing offer methods for assessing perceptions (Dawkins, 1999). Previous studies have implemented a variety of test arrangements, such as simple choice tests (Sanotra et al., 1995), varying cost motivational tests (Cooper and Appleby, 1997; Cooper and Appleby, 2003; Olsson and Keeling, 2002), operant condition tests with key pecking (Faure, 1994), approach-avoidance tests (Webster and Fletcher, 2004), and interconnected compartments (Albentosa and Cooper, 2005). These studies have reported preferences for environmental features, such as perches, nest boxes (Freire et al., 1996; Freire et al., 1997), dustbaths (van Lier, 1990; Sanotra et al., 1995), lighting (Davis et al., 1999; Prescott and Wathes, 2002), cage size and feeder space (Faure, 1986), as well as design and construction of cage furnishing. Preference and motivation studies have been used to demonstrate strong motivations for perches and nest boxes (Cooper and Appleby, 1997; Olsson and Keeling, 2002), which consequently led to changes in regulations for housing laying hens in the EU (EFSA, 2005).

Atmospheric ammonia is a common noxious gas in laying-hen housing with potential bird health and production implications (Faddoul and Ringrose, 1950; Anderson et al., 1964; Sato et al., 1973; Cottrell and Nordsog, 1954; Charles and Payne, 1966). Ammonia concentrations at ventilation exhaust from commercial egg laying facilities have been reported to range from 3 to 50 ppm for different housing systems and weather conditions (Wathes, 1998; Liang et al., 2005). Although these values are not necessarily reflective of concentrations experienced at bird level, they demonstrate a large variation. Limited information was found regarding hen preference for air quality. One study was found using a chamber for testing environmental conditions, reporting that hens find atmospheric ammonia concentrations greater than 25 ppm highly aversive (Kristensen et al., 2000). Another test was found for testing stun gases using approach-avoidance method (Webster and Fletcher, 2004).

The objectives of this work were: 1) to design and construct an environmental preference test chamber (EPTC) that enables the user to manipulate the environmental condition and monitor behavior of birds in response to the testing environment individually or in a group; and 2) to conduct an initial experiment to assess the performance of the EPTC and delineate aversion or preference response of laying hen to two levels of atmospheric ammonia.

## Materials and Methods

### *Environmental Preference Test Chamber (EPTC)*

The EPTC consisted of four interconnected compartments, each accessible to two adjacent compartments with a hanging door mounted in a connection passageway. The compartments were constructed with clear acrylic panel (6 mm or 1/4 in) and housed a wire-mesh cage divided into two sections, one for three stimulus birds to reside and the other for one test bird with access to the passageways (Figure 1a). Stimulus birds (3 per cage, 12 total) provided a group setting to avoid effects of isolation in preference tests. Each compartment provided 729 cm<sup>2</sup> (113 in<sup>2</sup>) floor area for the test bird and 1097 cm<sup>2</sup> (170 in<sup>2</sup>) per bird for the stimulus birds (Figure 1b). Each hanging door assembly (four total) consisted of three connection pieces, one mounted to each adjoining compartment and one connector that contained the hanging door. The connection pieces and hanging door (20 by 34 cm or 7.75 by 13.25 in, W by H, suspended

at top by two u-bolts 6 mm or 1/4 in diam.) were constructed of clear acrylic panel (6 mm or 1/4 in and 3 mm or 1/8 in, respectively).

The cages were raised above the compartment floor, and manure fell into a removable tray suspended beneath each cage. Each compartment provided handling access to stimulus hens via a wire mesh door fitted into one side wall of each cage and complementary hinged wall in the compartment. Access to test bird area was provided through the top of each compartment by removal of the inlet plenum box, affixed to the top by side latches.

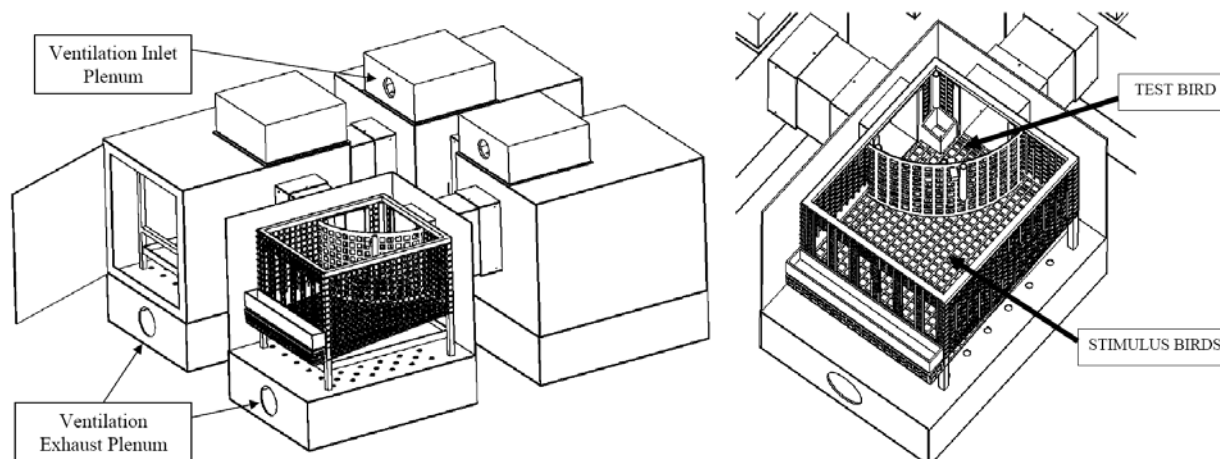


Figure 1: a) Left: schematic of environmental preference test chamber; b) Right: schematic of layout within one compartment.

### ***Ventilation and Air Handling***

Air was supplied to each compartment by individual fans mounted inside one of two insulated mixing boxes (Figure 2a). Fresh air was drawn into each mixing box near the ceiling. Two fin electric heaters (350W, Vulcan 0SF1510-350A, Cat. No. 3HM48, Grainger, Kansas City, MO) were suspended in the center of the mixing box, powered by a variable voltage supply, and connected with a temperature limit switch for safety. This allowed for control of supply air temperature, if desired. Two small mixing fans (12V DC mini-fan, Model 2730240, Radio Shack, Ames, IA) were located in the opposite corners near the top of the box and oriented diagonally to enhance mixing within the box. The supply air was delivered to each EPTC compartment through use of flanges (10 cm or 4 in dia., aluminum, RS-100, Maurice Franklin Louver Co., Georgetown, SC), a semi-transparent flexible hose (10 cm or 4 in dia., UFD.020 Thermo Polyurethane Flexible Duct, Item No. 48667, US Plastic Corporation, Lima, OH), and the ventilation supply fan (Delta FFB0412SHN, Cat. No. TGS10-12FAN, RaQware, Shreveport, LA) located at the inlet side of the flexible hose (mounted to the mixing box wall). For the ammoniated compartments, compressed  $\text{NH}_3$  (10%) was injected, as needed, into the supply duct approximately 5 cm (2 in) beyond the supply fan.

Each compartment featured a ventilation inlet plenum with an array of 61 holes (19 mm or 3/4 in dia. in an area 47 cm by 47 cm or 18.5 by 18.5 in) located above the test bird area. The ventilation air would pass the bird area, then through an array of 61 holes (2.54 cm or 1 in dia. in an area 67 cm by 67 cm or 27 in by 27 in) at the bottom of the compartment, and finally be exhausted to the ventilated room. Ventilation rate to each compartment was checked for uniformity prior to the experiment by assessing velocity in a cross-section of the supply hose and thus flow to each compartment, ranging from 9.3 to 10.5  $\text{m}^3/\text{h}$  (5.5 to 6.2 CFM) or amounting to approximately 19 ACH (air changes per hour).



Figure 2: a) Left: instrumentation for control system; b) Right: photo of complete environmental preference test chamber

### **Control Systems**

A Campbell CR10 measurement and control module (model CR10, Campbell Scientific, Inc., Logan, UT) was programmed to collect data and implement feedback to control the ammonia concentration within each compartment (Figure 2b, Figure 3). One air sampling line was located along the wire mesh divider within each compartment, with a stainless steel microfilter (5  $\mu\text{m}$  pores, Cat. No. 48222-02, MicroSolv Technology Corporation, Eatontown, NJ) at tubing inlet. An additional sample port was located near the ceiling of the room for sampling ambient air. The sampling lines were connected to one of four solenoid valves (24 VDC, Burkert, model # 456655, Wirrel, UK) controlled by relay switches (SDM-CD16AC, 16 Channel AC/DC Controller, Campbell Scientific, Inc.) for switching to sample air for each compartment. A diaphragm pump (Gast Linear SPP-6GAS-101, Cat. No. 79610, Cole-Parmer, Vernon Hills, IL) was used to deliver the sample air to the photoacoustic infrared ammonia gas monitor (Chillgard RT  $\text{NH}_3$ , Mine Safety Appliances Company, Pittsburgh, PA). Flow meters (0.5-5 LPM, RMA-26-SSV, Cat. No. 116273-30, Dwyer, Michigan City, IN) were used to control sampling rate, supply rate, and bypass for excess flow. Temperature and relative humidity (RH) were monitored within each compartment using T/RH sensors (HMP35C, T/RH probe, Campbell Scientific, Inc.) located along the divider within each compartment.

Ammonia was supplied to each compartment with compressed 10%  $\text{NH}_3$  balanced in  $\text{N}_2$ . The supply was controlled by individual mass flow controllers (0-100 sccm, FMA5508, Omega Engineering, Inc., Stamford, CT). Voltage supply to the mass flow controllers for feedback control was provided by voltage divider PC boards connected to additional channels on the relay board for the solenoid valves. An additional solenoid valve was located in the  $\text{NH}_3$  supply line to shut off flow in the event of a power failure.

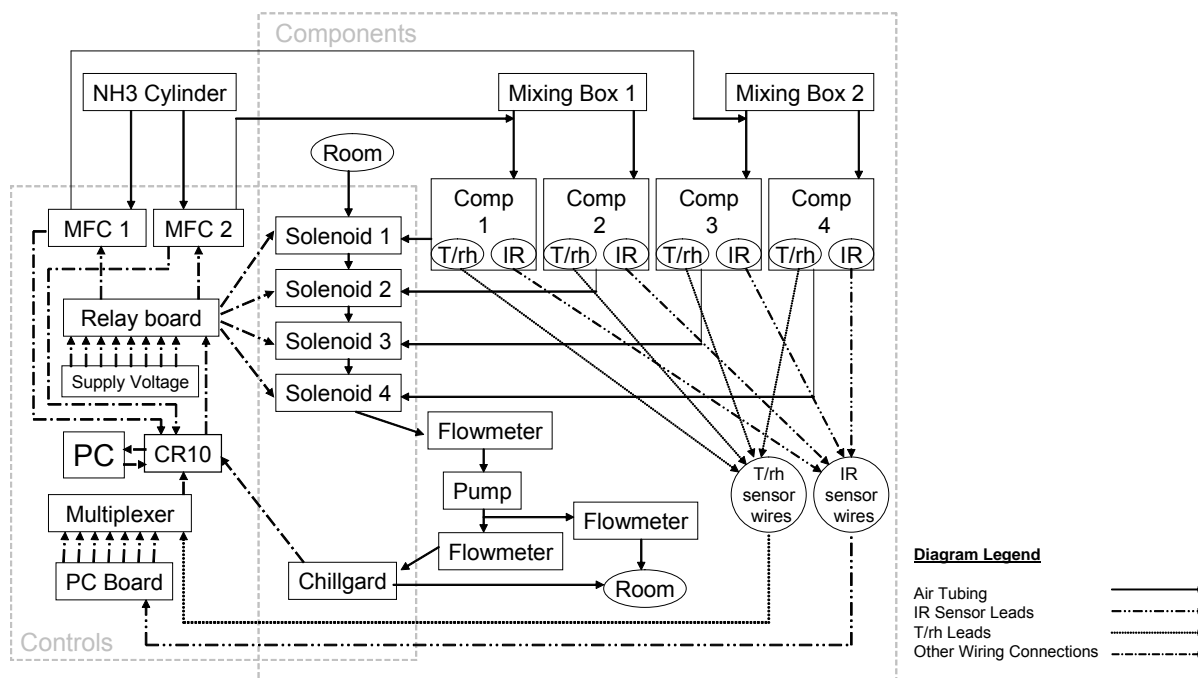


Figure 3: Block diagram of complete environmental preference test chamber system.

### Bird Tracking Systems

The EPTC was equipped with detection sensors to determine the location of the test hen within the chamber. Three IR emitter-detector pairs (5 mm, 890 nm, OP291A LED and OP555A Phototransistor, Cat No. 365-1057-ND and 365-1077ND, Digi-Key, Thief River Falls, MN) were mounted within each test bird area (Figure 4). Emitters were mounted into PVC tubing for protection of wires and placed above the feeder. Detectors were also mounted into PVC tubing and placed along the divider. Therefore, a hen standing in the test area would be blocking at least one pair. Sensors were connected to a PC board, powered with 2.5V, and the output voltage connected to the CR10 multiplexer (AM416 Multiplexer, Campbell Scientific, Inc.). The EPTC was also equipped with digital video monitoring and recording. One video camera was located above each test bird section, and recorded continuously for the duration of the trial.

### Experimental Hens and Husbandry

Hens (Hy-Line W-36) for this study were acquired from a commercial farm, and were previously used in an experiment assessing thermal environmental conditions under varying housing arrangements. At the time of preference data collection, the hens were 70 - 76 weeks old. All the experimental hens were acclimated under 21°C and <5 ppm ammonia environment for several weeks prior to placement in the EPTC. During this time, hens were housed in a holding cage fitted with an identical passageway and connected to an adjacent cage for training purposes. Test birds and most stimulus birds were housed in the same room with visual and vocal, but not physical, contact. Additionally, four stimulus birds (one for each compartment) were housed with and trained with the test birds.

During the preference test, feed was provided to the stimulus birds by a trough near the access door and to the test bird by a container located in the corner of each compartment. Nipple drinkers were located along the wire mesh divider in each compartment. Throughout the entire process, birds were allowed *ad lib* access to feed and water. Uniform lighting among the



compartments was achieved by placing the light at the center of the chamber, facing upward into the supply hoses (acting as a light diffuser).

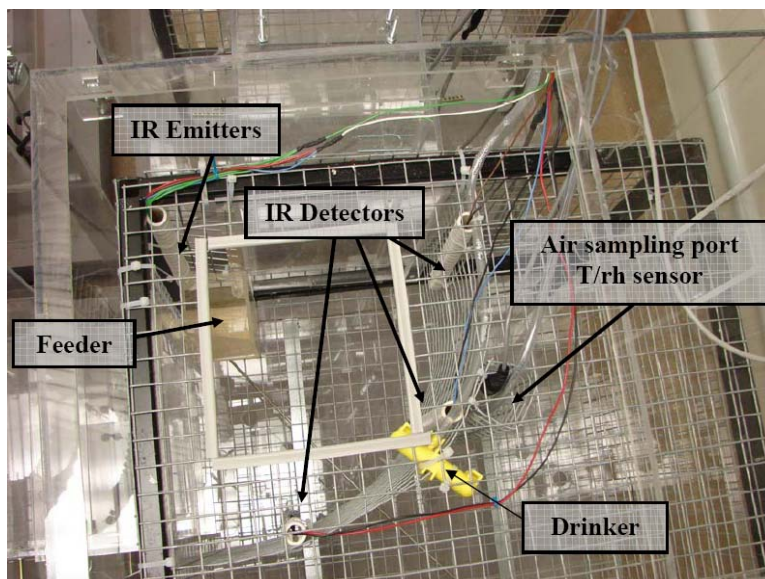


Figure 4: Top view of test hen area in one compartment.

### ***Training Birds***

Prior to the preference test, birds were trained to navigate the acrylic hanging door. Two holding cages were joined by a door fitting as described in the husbandry section. Initially, all birds were housed in the same holding cages. Twelve birds were selected from the holding area based on condition and appearance (likely dominant birds but not verified) and placed into the larger of the two cages. The hanging door was fastened open for two days. Several birds thoroughly explored both cages, and the door was returned to its hanging position. No additional incentive was offered, and within a few days, some birds had learned to navigate the door with ease. All other birds learned the task from these birds within a few weeks. All 12 birds learned to navigate the door, though only six were ultimately used in the experiment.

### ***Experimental Design***

Test birds were randomly selected from the trained birds, and assigned to the EPTC. Treatments were assigned to compartments in a randomized complete block arrangement, according to the treatment scheme outlined in Table 1. For the initial experiment, two ammonia treatments, 25 ppm and <10 ppm, were applied to each of two compartments simultaneously. Once the trial began, the test bird was given at least 1 day to acclimate to the EPTC under thermoneutral conditions (21°C) and ammonia level <2 ppm. During this period, the test bird was observed to demonstrate its navigation of the EPTC by moving into and out of each compartment at least one time. Following the acclimation period, bird behavior was collected for 2 days at comfortable conditions (21°C, <2 ppm NH<sub>3</sub>) and 3 days with ammonia treatment imposed. On the morning of the transition day between baseline and treatment, manure was removed, eggs were collected, and feed was replenished in all compartments. Following this, ammonia injection rate was increased hourly over 5 hours to achieve 25 ppm.

Table 1. Statistical design and treatment allocation, atmospheric ammonia concentrations less than 10 ppm (A<10 ppm) or approximately 25 ppm (A=25), for final 3 days of each trial. Baseline data in preceding 3 days were collected under essentially fresh air.

Trial	Compartment 1	Compartment 2	Compartment 3	Compartment 4
1	A<10	A<10	A=25	A=25
2	A<10	A=25	A<10	A=25
3	A=25	A=25	A<10	A<10
4	A=25	A<10	A=25	A<10
5	A=25	A<10	A<10	A=25
6	A<10	A=25	A=25	A<10

## Data Analysis

Total time spent in each compartment was analyzed with data collected by the automated tracking system. An algorithm, based on the IR sensor output, was developed to create a summary of location by time and calculate time spent in each condition. The processing algorithm was verified with videos for one 24 h period.

Location information was summarized into compartment occupancy for each day of each trial. Summaries were completed for complete 3 day baseline and treatment periods, as well as third (and final) day only of the baseline and treatment periods. Data summaries were compared in SAS PROC MIXED for effects of treatment, compartment, phase, and hen. An analysis was also completed using treatment and baseline differences, with the effect of phase removed. Effects were considered significant for  $\alpha < 0.05$ .

## Results

The EPTC design was completed, and the chamber was constructed. An initial experiment was conducted to assess hen preference for fresher air vs. polluted air (a lower  $\text{NH}_3$  vs. a higher  $\text{NH}_3$  concentration). Figure 5 shows IR sensor output and corresponding hen location for a sample dataset over several hours. Table 2 presents tracking system accuracy as compared to video analysis for hen occupancy and number of movements into each compartment. Differences in hen occupancy between the two methods were 0.0, 0.2, 0.1, and 0.0 h (for compartment 1, 2, 3, and 4, respectively). Total compartment entries calculated were 179 with the tracker and 242 with the video. Table 3 displays hen occupancy and number of moves into each compartment for complete 3 day baseline and treatment phases. There was no compartment effect for occupancy or number of entries for baseline or treatment phases. Table 4 lists occupancy and number of move into each treatment during the treatment phase only. There was no treatment effect on occupancy or number of entries (11.6 vs. 12.5 h or 45 vs. 47 entries for ammonia <10ppm vs. 25ppm, respectively). In general, the number of moves tended to be greatest on the first day and declined for each consecutive day of the trial. Similarly, there was a slight increase in number of moves on the first treatment day with subsequent decline. Figure 6 presents a sample of environmental conditions, compartment temperature and atmospheric ammonia concentration, for one complete trial, including 3 day baseline and 3 day treatment phase.



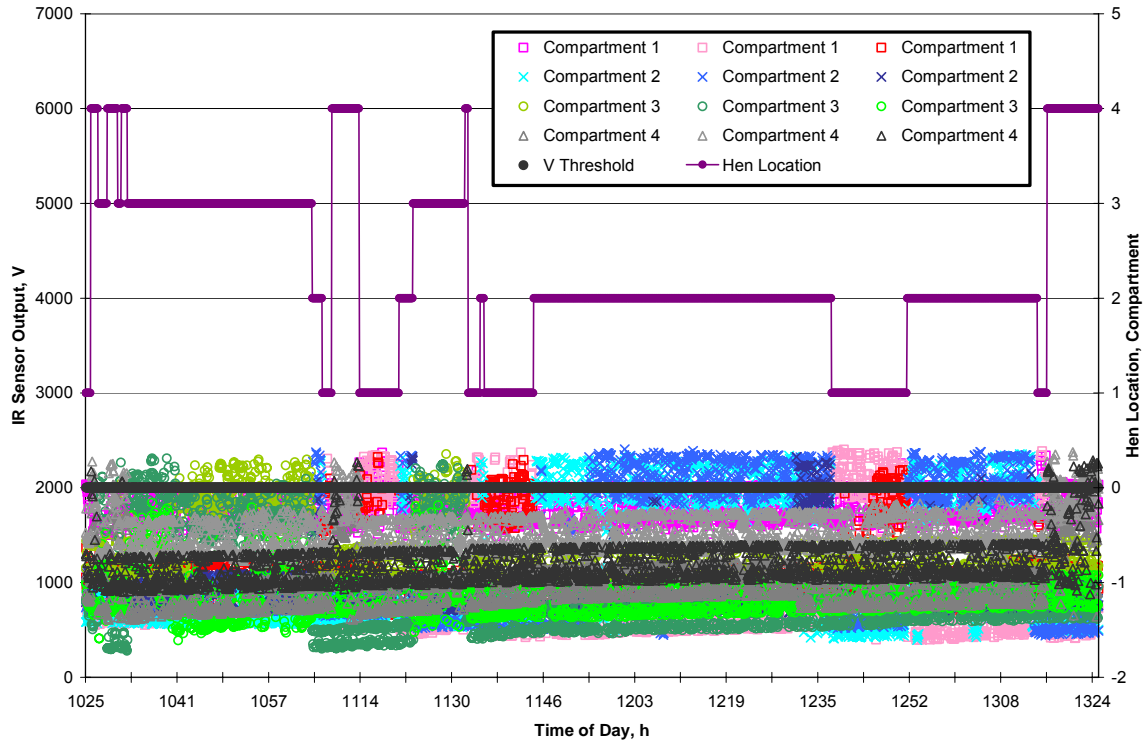


Figure 5: Comparison of IR sensor output vs. verified hen location in the laying-hen EPTC, with marked voltage threshold for data processing algorithm.

Table 2. Laying hen occupancy and entries into the EPTC compartments during a 24 h period, as calculated by automated tracking system (ATS) and video analysis (VA).

Comp	Cumulative Occupancy				Compartment Entries		
	ATS, h	VA, h	Difference, h	Difference, %	ATS	VA	Difference, %
1	2.4	2.5	0.0	0.9	48	61	21
2	4.2	4.0	0.2	4.7	50	61	18
3	10.6	10.8	0.1	1.3	38	60	36
4	5.9	6.0	0.0	0.5	43	60	28
					179	242	26

Table 3. Laying hen mean daily occupancy and entries into the EPTC compartments during 3 day baseline and 3 day treatment phases (n=6).

Comp.	Occupancy, h		Occupancy, %		Compartment Entries	
	Baseline	Treatment	Baseline	Treatment	Baseline	Treatment
1	5.5	5.8	22.7	24.1	23	22
2	7.9	8.8	32.6	37.0	27	26
3	6.3	5.5	26.1	23.0	19	14
4	4.6	3.8	18.6	15.9	23	20
SE	1.0	1.0	5.0	5.0	6	6

Table 4. Laying hen mean daily occupancy and entries into the EPTC compartments with ammonia level of <10 ppm or 25 ppm during the final 3 days of preference trials (n=6).

Treatment	Occupancy, h	Occupancy, %	Compartment Entries
A<10	11.6	48.0	45
A=25	12.5	52.0	47
SE	2.0	10.0	12

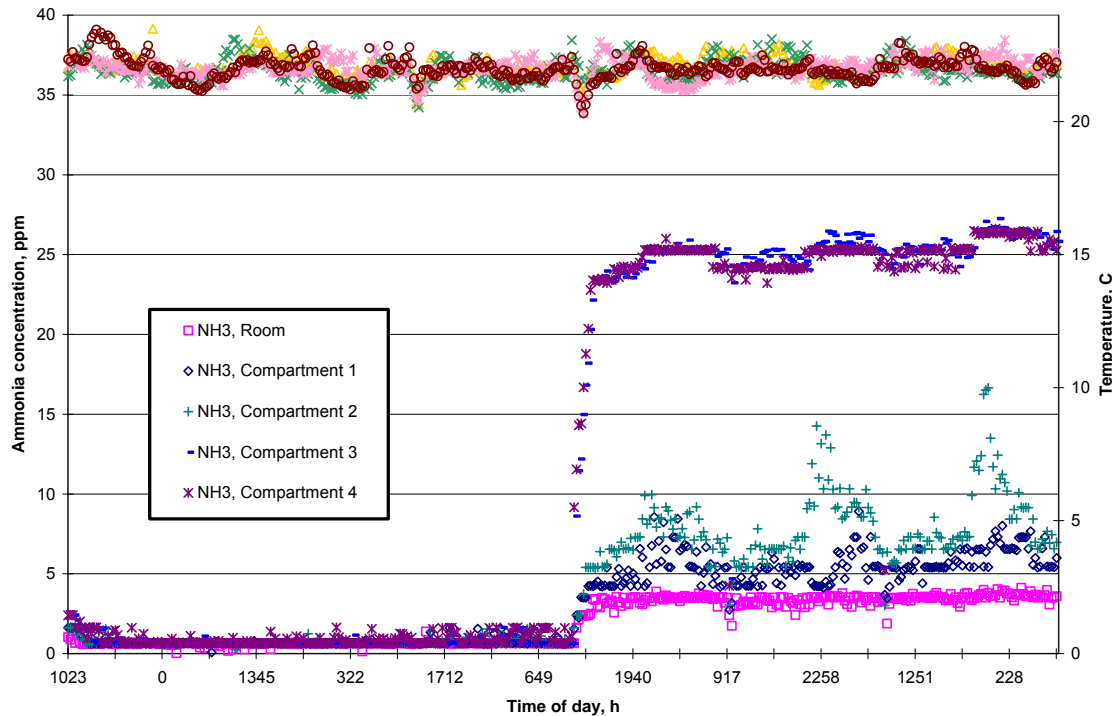


Figure 6: Sample compartment environments (during one complete trial) for baseline and treatment phases (3 day each) within laying hen environmental preference chamber.

## Discussion

The EPTC described in this study is different from previous chambers (Kristensen et al., 2000; Webster and Fletcher, 2004) in that it allows for collection of individual behavior without effects of social isolation. It would be expected that the stimulus birds may affect hen choices, but this effect should be included in the compartment effect (since stimulus birds were always located in the same compartment) and would be overcome by proper randomization and replication. Another benefit is that the divider can be removed if group behavior is desirable, or to supplement individual behavior results.

Prior experience affects subsequent choice (Dawkins, 1976; Bradshaw, 1992). In a no-cost vs. cost preference test, access to six areas was offered from a central empty wire mesh cage. Addition of the cost (squeezing between two vertical rods at the door) resulted in decreased frequency of movements, but did not decrease the time spent scratching and pecking, indicating an ethological need (Bradshaw, 1992). A similar approach can be implemented by varying the weight of the door in the EPTC.

The design of the EPTC included allowance for testing synergistic effects of environmental factors, such as varying temperature and atmospheric ammonia levels simultaneously, which should be explored in future experiments, as discussed below. There are many other additional applications of the chamber, ranging from air quality to environmental enrichment to nutrition.

Few problems were encountered in the initial application of the EPTC. The most critical challenge resulted from cross-contamination of  $\text{NH}_3$  into “fresh air” compartments from the ammoniated compartments. This likely resulted from the variation in ventilation rates from compartment to compartment, which were slightly different at the start of the trials (18-19 ACH) but likely varied as dust accumulated on the exhaust filters. Feedback could be added to the voltage source to the supply fans to adjust ventilation rate, or adjustable dampers could be added.

The tracking system correctly identified the majority of entries into compartments (179 out of 242 entries or 74%). Quick entries and exits were not recorded due to the sampling rate of the sensors (5 s), but the sampling frequency can be increased in future studies if capture of these quick passages is critical. Because the duration of these entries was short, the results for compartment occupancy were only slightly impacted by the shortfall to identify quick moves (maximum of 4.7% difference for one compartment). This likely became less important as trials progressed because the number of moves tended to decrease as the trial progressed, with the most on the first day presumably due to chamber exploration. Further validation of the tracking system performance should be completed using more than one day of data and multiple birds. Optimization of the algorithm used to assess hen location might further reduce occupancy error.

The lack of aversion to atmospheric ammonia observed in this initial study contradicts results reported by Kristensen et al. (2000) and could have resulted from several factors. It is possible that the hens did not find the concentrations in this study aversive. The age of the hens and previous exposure to atmospheric ammonia may have reduced their ability to detect it or may have increased their tolerance level. Mature hens (70 to 76 weeks old) were used in this experiment, and were 40 weeks old when acquired from high-rise houses on a commercial farm during winter. It is possible that genetics of the hen could also affect perceptions. It is also possible that the hens became desensitized to the ammoniated compartments after initial exposure; though this is not likely based on the results of a neurological study quantifying the nerve responses to short-duration ammonia exposure (McKeegan et al., 2002). The previous study used a brown variety, whereas this study used a white leghorn (W-36). Another possibility is that the hen's desire to remain with a particular social group or interact with all social groups outweighed the desire for fresher air. Because of individual bird to bird variability, a sample size of six birds may be insufficient to reveal an actual aversion. It also must be considered that the hens were unable to associate compartment with environment, and therefore did not recognize the choice offered. One previous study reportedly overcame this obstacle by placing color markers within compartments (Abeyesinghe et al., 2001). These possibilities should be further explored before making conclusions based on the results of this experiment.

Further analysis of the data collected may yield more insight to the perceptions of the hens in this experiment. Occupancy data may be extracted for photoperiod. None of the hens moved during the dark period, heavily weighing occupancy toward the night compartment. Compartment usage during light period may yield different results than total occupancy. In addition, further analysis should include correlations of behaviors with location and environment. An ethogram should include the following behaviors and postures: eating, drinking, sitting, standing, traveling, preening, interacting with conspecifics, other, and unknown/out of view. Behavior and occupancy data may be supplemented with location of egg-lay and quantification of feed disappearance, feed wastage, and manure dispersal in each compartment.

Assessment of hen environmental perceptions should not be over-simplified or over-generalized. Limitations of preference testing and interpretation of results are acknowledged (Duncan and Dawkins, 1977; Hughes, 1977). It must be considered that some preferences may be non-exclusive, prefer to do a certain behavior in one space and a different behavior in another space (Nicol, 1986). It has also been observed that preferences do not always correlate with functionality. For example, hens were shown to prefer open-sided cages over solid-sided cages (Elston et al., 2000a), even though no behavioral differences were observed within the two types (Elston et al., 2000b). A thorough exploration of methods should be implemented before conclusions are drawn.

## Conclusions

An environmental preference test chamber (EPTC) for laying hens was successfully designed and constructed. The chamber consists of four interconnected compartments with an area for a test bird to navigate between the compartments and an area in each compartment for a group of three birds to reside. The EPTC incorporated automated environmental control for atmospheric ammonia concentration and an automated tracking system for location of the test bird. An initial experiment was conducted using the EPTC to assess aversion of laying hen to atmospheric ammonia. The automated tracking system yielded less than 5% error for compartment occupancy, but failed to identify quick moves through compartments due to sensor sampling rate. Results from the initial hen ammonia aversion assessment warrant further investigation.

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